

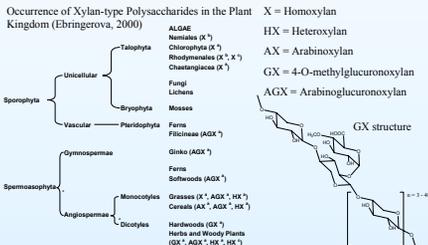
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## Occurrence

- Xylans occur in many structural varieties in terrestrial plants.
- GX and AGX are the main structural xylans in biomass feedstocks.



## Importance

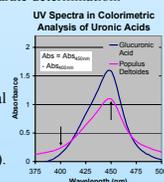
- Hardwoods contain 15-30% of hemicellulose which is predominately O-acetyl-4-O-methylglucurono- $\beta$ -D-xylan.
- The xylose : 4-O-methylglucuronic acid ratio varies considerably with species and plant part from 1 – 20 : 1 (Ebringerova, 2000).
- Whereas the acetyl and xylosidic bonds are easily cleaved the uronic acid - xylose bonds are very resistant to acid hydrolysis.
- Softwoods and herbaceous plants contain arabinoglucuronoxylan. The glycosiduronic bonds in AGX are very resistant. (Sjostrom, 1981)
- 2-O-(4-O-Methyl-glucopyranosyl)uronic acid - xylopyranose is hydrolyzed 20x slower than corresponding xylitol (Timell, 1964)
- Cellobiouronic acid is hydrolyzed 30x slower than cellobiose.
- Because of the resistance of the uronic acid – xylose linkage to acid hydrolysis a fraction of the xylose is not released in dilute acid pretreatment of biomass feedstocks.

## Total Uronic Acids Analysis Decarboxylation

- Biomass sample decarboxylated by boiling HCl (12%). CO<sub>2</sub> adsorbed in ascarite and quantified by weight gain. Decarboxylation can take more than 4 hours. (Browning, 1949)
- Decarboxylation by reflux in HI. Liberated CO<sub>2</sub> absorbed in NaOH. CO<sub>2</sub> quantified by change in conductance of NaOH. Suitable for 50 – 100 mg samples containing 1-10 mg uronic acid. (Theander, 1991)
- CO<sub>2</sub> produced from non-uronic extractives and carbohydrates complicates analysis. Even after extraction of biomass decarboxylation gave 30 – 40% higher uronic anhydride contents than colorimetry method. (Scott, 1984)

## Total Uronic Acid Analysis Colorimetric Determination

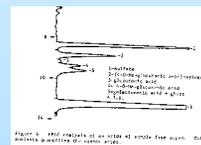
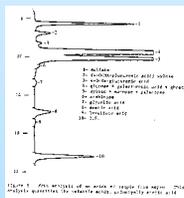
- Hydrolyzates reacted with conc. H<sub>2</sub>SO<sub>4</sub> at 70 °C converting uronic acids to 5-formyl-2-furoic acid, which is colorimetrically determined after reaction with a phenol, e.g., 3,5-dimethyl phenol
- Glucuronic acid reacts much more slowly than other uronic acids. Addition of boric acid allows its separate determination.
- Presence of lignin requires subtraction of reagent-less blank
- Different factors used depending on uronic acids present
- Reaction/measurement timing critical
- IEA round robin found 20 – 30% between-lab reproducibility in 4 feedstocks tested (UA content 1-4%). (Scott, 1979; Agblevor, 1992)



## Analysis of Individual Uronic Acids Cation HPLC

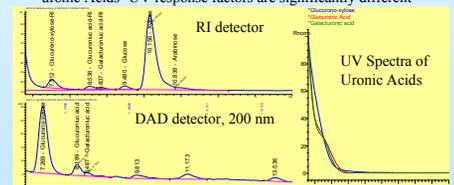
- HPLC of hydrolyzates on Bio-Rad HPX-78H column allows separation of 3 uronic acids and the aldoiburonic acid. (Kaar, 1991)
- Neutral sugars are removed from hydrolyzate using H<sup>+</sup> cation-exchange resin in a 7-step procedure.

- Losses of uronic acids during hydrolysis (30 – 40%) not factored into calculations
- Refractive Index detector used



## Analysis of Individual Uronic Acids Cation HPLC

- Uronic acids absorb in the UV from 190 – 240 nm.
- With DAD detector sugar removal no longer necessary.
- Glucurono-xylose standard needed as Glucuronic and Galacturonic Acids' UV response factors are significantly different



## Analysis Standards

- 4-O-methyl-glucuronic acid (MeGA) and 4-O-methyl glucurono-xylose are not commercially available.
- MeGA has been synthesized from methyl glucoside. Benzoylation is followed by methylation at the 4-position, debenzoylation, and then oxidation with TEMPO. Overall yield (74%) (Li, 1995).
- GX and AGX hemicellulose have been extracted with alkali from biomass samples and holocellulose (Timell, 1964; Ebringerova, 2000). Partial acid hydrolysis should yield 4-O-methyl glucurono-xylose.
- Without good standards it is hard to believe that much progress will be made in developing a routine analysis.

## Conclusions/Future Work

- A routine method for analysis of the individual uronic acids is needed if we are to track the fraction of xylose not released in acidic biomass pretreatment.
- So far either cationic or anionic HPLC appears the most promising, however, without good standards accurate analytical data will not be possible.
- A synthesis of MeGA is underway at NREL but has not yet yielded the desired compound.
- Isolation of the uronic acid – xylose from GX extracted from biomass will also be attempted.

## Acknowledgements

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